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Methodological Approaches to the Study of the Combined Effect of Atmospheric Pollutants as Illustrated by Chlorinated Hydrocarbons

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The combined effect of substances with a unidirectional toxic action was studied with the use of several chlorinated hydrocarbons, including 1,2-dichloropropane, 1,2,3-trichloropropane, and perchlorethylene at various concentrations.

The results of the study can be used as the basis for the development of methodological approaches to the study of the combined action of toxic substances administered by inhalation.

The research plan also incorporated the problem of explaining the nature of the action of the mixture of the test compounds in acute, subacute, and chronic animal experiments. All of the studies were conducted with each compound alone and then in various combinations by using the concentration—time relationship, since a correct evaluation of the combined action of compounds can be obtained by comparing these with the effects observed for the action of each component alone.

A graphic representation was obtained of the onset time for a given effect (loss of righting reflex for 50% of the animals) as a function of the active concentrations for the short-term effect of high concentrations of 1,2-dichloropropane, 1,2,3-trichloropropane, and perchloroethylene based on the concentration—time relationship. The nature of the combined action of various combinations (two and three substances) with respect to concentration was studied by using these graphs.

At high concentrations, the action of various combinations of the studied substances is additive. An inverse relationship was discovered in subacute experiments between the inactive concentrations and the onset time for changes in the vital activity indices of an organism: the lower the concentration, the later the changes.

Additional data were obtained as the result of morphofunctional studies on the toxic properties of the studied compounds, on damage reactions and on adaptational changes. This is of interest from the point of view of explaining the mechanism of action at various concentrations.

Ever-increasing development of industry, agriculture and transportation produces unavoidable pollution of atmospheric air by multicomponent mixtures of chemical compounds.

In reality, man is not exposed to isolated substances, but to mixtures of substances, and therefore study of the effect of the environment on organisms must begin with the analysis of the effect of individual factors with subsequent study of their combinations.

However, because of the complexity of the problem from the methodological point of view as

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well as the laboriousness of such studies, especially on multicomponent mixtures, work on the nature of the combined effect of chemical substances occupies a minor place in the arsenal of toxicological research.

The evaluation of the combined effect of atmospheric pollutants is particularly complex. However, the factors complicating the study of this problem should not be allowed to stand in the way of its solution. Otherwise, it will be impossible under current conditions to evaluate environmental pollutants correctly and to develop effective health measures.

It is known that in the case of the simultaneous combined effect of an organism, the degree of toxicity of substances may vary in the direction of increase, summation or weakening of effect.

McGowan (*1*) and Ball (*2*) considered the action of nonelectrolytes to be additive. Elkins (*3*) also believes that substances related to one group have an additive effect, but an additive effect for compounds of different groups is doubtful.

Other authors (*4,5*) have noted differences depending on the quantity and time of effect of a toxin. Therefore, with extended effect on a toxin in small concentrations, the phenomenon of potentiation noted in acute experiments is not observed.

According to data of Shtessel (*6*), Lyublina (*7*), Ulanova and Zayeva (*8*), and Sanotskii (*9*) the effect of narcotic substances of one group is usually additive at both the lethal and threshold concentration levels in a chronic experiment.

Analysis shows that the results of studies of the nature of the combined effect of toxic substances are contradictory. The applicability of extrapolation from high concentrations to those low ones encountered in atmospheric air, requires specific studies. The nature of the effect of combinations of various substances must be related to the mechanism of their toxic effect and the active concentrations.

In accordance with the joint U.S.–U.S.S.R. research program research has been conducted at our Institute related to the combined effect on an organism of several compounds of a group of chlorinated hydrocarbons, namely, i.e., 1,2-dichloropropane, 1,2,3-trichloropropane, and perchloroethylene (tetrachloroethylene), at various concentrations. These substances are used widely in industry, chiefly as starting compounds and intermediates in the synthesis of various classes of organic compounds, as well as in agriculture where thy are used as insecticides. Information concerning their toxic properties is limited. 1,2-Dichloropropane has been studied comparatively well.

Substances of this class produce marked changes in the central and peripheral nervous systems, degenerative changes in parenchymatous organs—mainly in the liver–and blood dyscrasias (*10–18*).

In planning research, we set ourselves the task of explaining the nature of the effect of mixtures of the studied substances under conditions of acute, subacute, and chronic experiments. It is known that correct evaluation of the combined effect is based on comparison of the effects produced by a mixture of substances and the effects observed in the case of the activity of each component of the mixture alone. Therefore, research was conducted with each substance individually and then in various combinations. We considered it possible to study the nature of the effect of mixtures of substances (at various concentration levels) on the basis of general principles of effects of chemical substances on an organism, particularly on the basis of the concentration–time relationship.

In acute experiments with white mice of uniform weight, we conducted dynamic inhalation exposures at the following concentrations: 1,2-dichloropropane, 26.0, 22.0, 17.5, 12.6, and 4.8 mg/l.; 1,2,3-trichloropropane, 6.6, 5.0, 4.1, 2.1, and 1.0 mg/l.; perchloroethylene, 33, 30.3, 18, 15.6, 11.5, and 5.2 mg/l.

The pattern of signs of intoxication was studied during exposure. The clinical pattern of intoxication was monotypic and characterized at first by symptoms of general agitation and decreased coordination of movements, followed by sluggishness, amyotonia, and sporadic clonic spasms, followed by loss of the righting reflex. The time of onset of the loss of righting reflex was accurately established in each experiment. The average onset time for loss of righting reflex of 50% of the animals (ET_{50}) was computed in accordance with the Bliss-Litchfield method (*19*).

Direct onset time relationships were obtained as a function of the effect of the concentration for each of the studied substances. These were plotted logarithmically.

In acute experiments we studied the combined effect of mixtures of 1,2-dichloropropane and perchloroethylene, 1,2-dichloropropane and trichloroethylene, and then of the three substances together (12 combinations in all).

From a graph of the concentration–time relationship for each of the studied stubstances for determination of the nature of their combined effect, an experiment with any mixture may be conducted (within the concentrations range tested for the isolated effect of substances) without making up a mixture of preset concentrations (definite dosage of concentrations in a mixture is usually very difficult). From a known concentration of each ingredient of the mixture and the computed average effective time (ET_{50}) of onset of loss of righting reflex in the animals, isoeffective concentrations of each component, i.e., those concentrations which produced a fixed effect during the same time from the beginning of the experiment as the mixture could be determined from the graph. We took each of the isoeffective concentrations as 100% or 1.0 and expressed the concentration of each ingredient of the mixture as fractions or parts of the corresponding concentrations producing a certain effect for an equivalent time when the substances were tested alone.

Mixtures with additive concentrations indices expressed as the sum of fractions of isoeffective values of each component were in the range 0.98 to 1.1 (93–110%). Therefore it may be considered that at a high concentration level, the effect of the studied substances occurs in an additive manner.

Subsequent experiments with lower concentrations and increased periods of exposure were also proposed. As in the first research stage, we first studied the effect of each substance individually and then combined effect of a mixture of substances. As the fixed effect we adopted the first reliable change in the physiological and biochemical indices used in the experiment in comparison to a control group. The time of these changes was strictly established.

Continuous inhalation exposure of white male rats weighing from 200 to 240 was carried out in 200-1. chambers for 7 days with the following concentrations: 1,2-dichloropropane, 2 and 1 mg/1.; 1,2,3-trichloropropane, 0.8 and 0.35 mg/l.; perchloroethylene, 2.75 and 1.25 mg/l. Each series of exposures had its own control group. We studied the following indices of vital activity: activity of acetylcholinesterase and blood catalases, content of red blood cells, white blood cells, and hemoglobin, summation threshold index, and weight of animals.

With the aid of the above-mentioned tests we studied the vital activity of organisms after 2, 4, 24, 48, 72, and 96 hr and after 6 and 7 days of exposure.

In evaluating the nature of the effect of the substances, we used functional morphology methods, including histostructural analysis of the liver and kidneys, determination of ribonucleic acid by the Brash method (with control of ribonuclease), glycogen by the Hotchkiss method (with control of amylase), staining with sudan black for determining lipids; oxidation process activity (succinic dehydrogenase according to the Nakhlas method), DPN–diaphorase by the Bernston method, and hydrolytic enzymes acid and alkaline phosphatase (using the Gomori method) as well as quantitative evaluation of liver DNA by a cytophotometry method.

Layer preparations of friable subcutaneous connective tissue were also studied, as systems of fat cells serve as integral indicators of a condition of homeostasis.

The initial verifiable changes in catalase activity, cholinesterase, and the ultimate threshold index, were observed 4 hr after the inhalation of dichloropropane and trichloropropane at concentrations of 2 and 0.8 mg/l., respectively, on animals in comparison to the control group. At a later period (72 hr) changes in these same indices appeared on exposure to perchloroethylene at a concentration of 2.75 mg/l.

Changes were observed in the above tests after 24 hr for the with dichloropropane at a concentration of 1 mg/. and trichloropropane at a concentration of 0.35 mg/l. In the case of perchloroethylene at a concentration of 1.25 mg/l., verifiable changes in these indices were evoked after 144 hr.

Other tests used in the experiment indicated only tendencies toward change at various times during the exposure of the animals.

The toxic effect was observed after a relatively short time. It was necessary frequently to study the functional state of the organism by observing selected indices to establish precisely the time of onset of changes in the animals. The larger number of tests used in the dynamic studies makes this difficult.

It seems advisable to discuss some of the results of morphological and histochemical studies to understand better some aspects of the toxic effect of the studied substances under conditions of their individual and combined action at various concentrations.

Analysis of morphological material confirmed that the studied substances produce a complex of structural changes of a nonspecific character in organs and tissue accompanied by disturbances in macromolecules and enzymes.

The degree of morphological changes varies with the concentration and nature of the substance. Toxic effects of trichloropropane and dichloropropane are much more marked than those of perchloroethylene. Liver and kidneys show great sensitivity. These organs show an ambiguous reactivity of endothelial cells to chemical effect in relation to their localization.

Maximum histostructural damage to the liver, accompanied by signs of protein-fat dystrophy, suppression of enzymic activity, and reduction of content of ribonucleoproteins, is found in the centrolobular sections, accompanied by distinct microcirculatory disturbances and increased vascular permeability (Figs. 1 and 2). Cells of peripheral sections of lobules show fewer changes and undergo displacements of an adaptational nature in the form of hyperplasia and hypertrophy of cellular and intracellular structures.

A verifiable increase is observed in the number of unicellular polyploidal hepatocytes with respect to the effect of dichloropropane and trichloropropane as the number of binuclear cells is reduced. Under these conditions, the number of cells with ploidy is equal to 16*p* (Fig. 3). Trichloropropane and dichloropropane produce a wave of mitosis, and mitosis of binuclear cells leads to appearance of two mononuclear cells of the same ploidy. These adaptive changes are accompanied by increased content of ribonucleoproteins and increased activity of enzymes on the periphery of

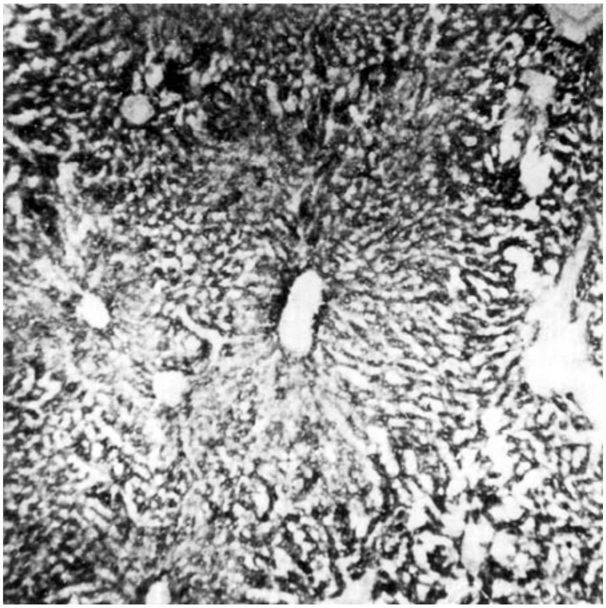

FIGURE 1. Succinic dehydrogenase activity in the liver. Control group, 150X.

In the kidney, as in liver, regions of greater and lesser sensitivity to chlorinated hydrocarbons were found. Most sensitive were the proximal convoluted tubules, ascending sections of the loops of

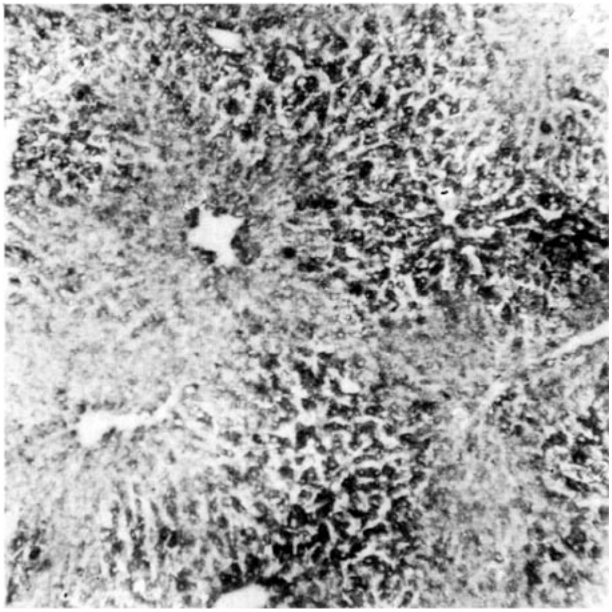

FIGURE 2. Reduction in succinic dehydrogenase in the central sections of the liver lobules as a result of exposure to 1,2-dichloropropane, 150X.

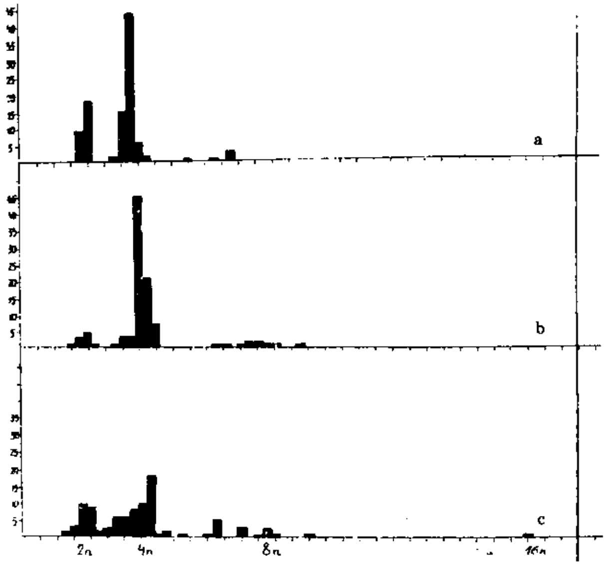

FIGURE 3. Effect of dichloropropane on rat liver cell ploidy: (a) control; (b, c) histograms of rat liver ploidy, from rats under the effect of dichloropropane.

Henle, and the collecting tubules (Yukst canals) of the medullar zone, for which histostructural changes accompanied by suppression of the activity of oxidation enzymes and phosphomonoesterase are characteristic. In distal segments of the nephron is found compensatory reorganization in the form of increased content of large molecules and increased enzymic activity.

Thus, the toxic effect of chlorinated hydrocarbon derivatives is reflected in histostructural, macromolecular and bioenergetic changes in liver and kidneys, accompanied by microcirculatory displacements and reactivity of stromal elements. In certain regions of these organs there develop damage reactions and adaptational shifts, relative to severity of intoxication. The region in which damage occurs is apparently linked to accumulation of studied substances and their toxic metabolites in various parts of the organs. Our hypothesis was confirmed by the study of Reid and Krishna (*20*) showing maximum accumulation of tracer halogenated hydrocarbons in the central parts of liver lobules, where the highest activity of microsomal enzymes carrying out metabolic transformation of lipotropic chemical substances is found (*21*).

The study of loose subcutaneous connective tissue preparations made it possible to explain the increased functional activity of the fat cell system in the case of the effect of all of the studied substances. This is expressed by a higher index of degranulation and by higher alkaline phosphatase activity in these cells (Figs. 4 and 5). We regard this integral reaction as reflecting adaptive shifts toward homeostatic equilibration. In addition, we observed the formation of degenerative forms and the decomposition of fat cells as the result of damage from chemical intoxication. This process is most marked in the case of rats which had been subjected to the effect of trichloropropane.

Conclusion

The short-term action of high concentrations of 1,2,-dichloropropane, 1,2,3-trichloropropane, and perchloroethylene was analyzed graphically by plotting the time of onset of a certain effect (loss of righting reflex in 50% of animals) against the active concentrations. We used these plots in studying the nature of the combined effect of two- and three-component mixtures in various concentrations.

At high concentrations, the effect of various combinations of the studied substances is additive.

A definite relationship was found (in subacute experiments) between active concentrations and time of onset of changes associated with the vital

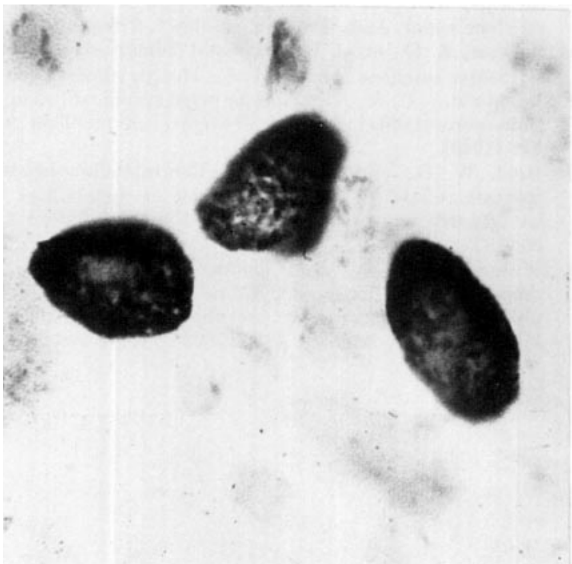

FIGURE 4. Fat cells for control animal. Staining with toluidine blue, 1350X.

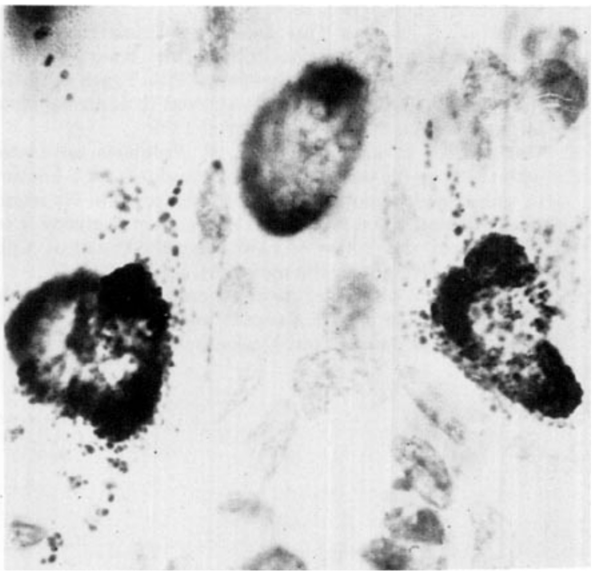

FIGURE 5. Degranulating fat cells of test animal exposed to 1,2- dichloropropane, 1350X.

activity indices of organisms: the lower the concentration, the later the changes occurred.

As the result of morphofunctional research we obtained supplementary information concerning the toxic properties of the studied substances, damage reactions and adaptation shifts. This is of interest from the point of view of explaining the mechanism responsible for the effect of substances at various concentration levels.

Future studies of each substance individually will make it possible to obtain a graph for the onset time of certain changes in indices as a function of active concentrations. On this basis it will be possible to determine the nature of the effect of a mixture in both subacute and chronic experiments.

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